MOLTING AND CYCLIC ACTIVITY IN CHROMATOPHOROTROPINS OF THE CENTRAL NERVOUS SYSTEM OF THE BARNACLE, BALANUS EBURNEUS [1]

JOHN D. COSTLOW, JR.

Duke University Marine Laboratory, Beaufort, North Carolina

Although molting in the acorn barnacles is more frequent than that described for most Crustacea, every 2–3 days in the warm-water species, and continues throughout the life of the individual barnacle (Costlow and Bookhout, 1953, 1956), nothing is known of the mechanisms which control the sequence of molting or the duration of the intermolt period (Carlisle and Knowles, 1959). Barnes and Gonor (1958a) have suggested that the control of molting in the Cirripedia may depend on the absence of a molt-inhibiting hormone and thus be more similar to the mechanism described for *Lysmata seticaudata* (Carlisle, 1953a, 1953b, 1953c; Carlisle and Dohrn, 1953) than the mechanism described for most decapods which is thought to involve the interaction of a molt-accelerating and a molt-inhibiting hormone (Passano, 1960, 1961).

Several attempts have been made to establish a relationship between cyclic activity of barnacle shell and body tissues and the regular sequence of molting periods. While cyclic activity has been described from a few tissues in barnacles (Thomas, 1944; Costlow, 1956), the activity has not been shown to be directly associated with either ecdysis or its control.

The present study has had two major objectives: (1) to determine if the *Uca* black-pigment-dispersing substance of the central nervous system of acorn barnacles (Sandeen and Costlow, 1961) is cyclic in its activity; and (2) if activity of this chromatophorotropin is cyclic, to determine whether the changes in activity are associated with the molting cycle of the adult barnacle.

Methods

Adults of *Balanus eburneus* were collected from pilings in the area of the Duke University Marine Laboratory and placed in the running sea water system. When it had been determined that the animal had not been damaged in the course of removal from the pilings, the barnacles were segregated into plastic compartmented boxes, placed at a slight angle under the direct flow of the sea water outlet, and fed *Artemia* nauplii. The barnacles were checked at regular half-hour intervals during the day and if a molt were found, the time was recorded and the animal removed to another container in running sea water.

At the intervals of time after molting shown in Table I the barnacle body was removed from the shell, dried on filter paper, and weighed to the nearest 0.1 mg. on a Roller-Smith balance. The central nervous system was then dissected intact

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TABLE I

Number of adult Balanus eburneus from which central nervous systems were extracted at intervals following molting and the dilutions of extract (CNS/ml) used

	Intervals Hours after molting					
Dilution	0	12	24	48	72	96
1:1	12	12	12	11	8	9
1:5	19	—	20	10	9	9
1:10	9	—	9	—	—	—

from the body and thoroughly triturated in a glass dish containing as little water as possible. The ground material was suspended in the desired amount of filtered sea water, boiled, and centrifuged.

Fiddler crabs, *Uca pugilator*, which had been destalked 12 to 24 hours previously, were then injected with the barnacle central nervous system extract. The standard dose was 0.05 cc., injected into the ventral hemocoel at the base of the fifth walking leg with a tuberculin syringe and a 26-gauge needle. Three different concentrations of extract were used: one central nervous system per one ml. of sea water, one per five ml. and one per ten ml. For control injections, homogenized opercular muscle from the barnacles was used and 5 fiddler crabs were injected daily with *Uca* eyestalk extract, one pair per 5 ml., to determine the variability of the bioassay animals as well as the technique.

Five eyestalkless fiddler crabs were injected with the extract from each central nervous system and the chromatophores of each animal staged at intervals of 15, 30, 45, 60, 90, 120, and 180 minutes following injection. The chromatophore scale of Hogben and Slome (1931) was used to determine the *Uca* chromatophore response to the barnacle central nervous system extracts. From the average chromatophore values for each reading the total net activity was determined for the entire 180-minute period (Sandeen and Costlow, 1961). All experiments were begun between 1 and 2 PM and terminated before 5 PM to avoid possible fluctuations in the crab chromatophores due to diurnal rhythms.

RESULTS

Figure 1 gives the average total activity values for extracts of *B. eburneus* central nervous systems removed at regular intervals following ecdysis and assayed on eyestalkless fiddler crabs. At a concentration of 1:1 the central nervous system had a low activity immediately following molting, followed by an increase in activity at 24 hours after molting. Statistical treatment of the data from these experiments indicates that variability in activity of the extracts within the sample is as high as that between the samples, and that the fluctuations in central nervous system activity at different periods of time after ecdysis are not significant at a concentration of 1:1.

Injections of the barnacle central nervous system extract at a dilution of 1:5 showed similar changes in the activity of the chromatophorotropins in relation to the molting cycle: a low point of activity immediately following molting, a peak

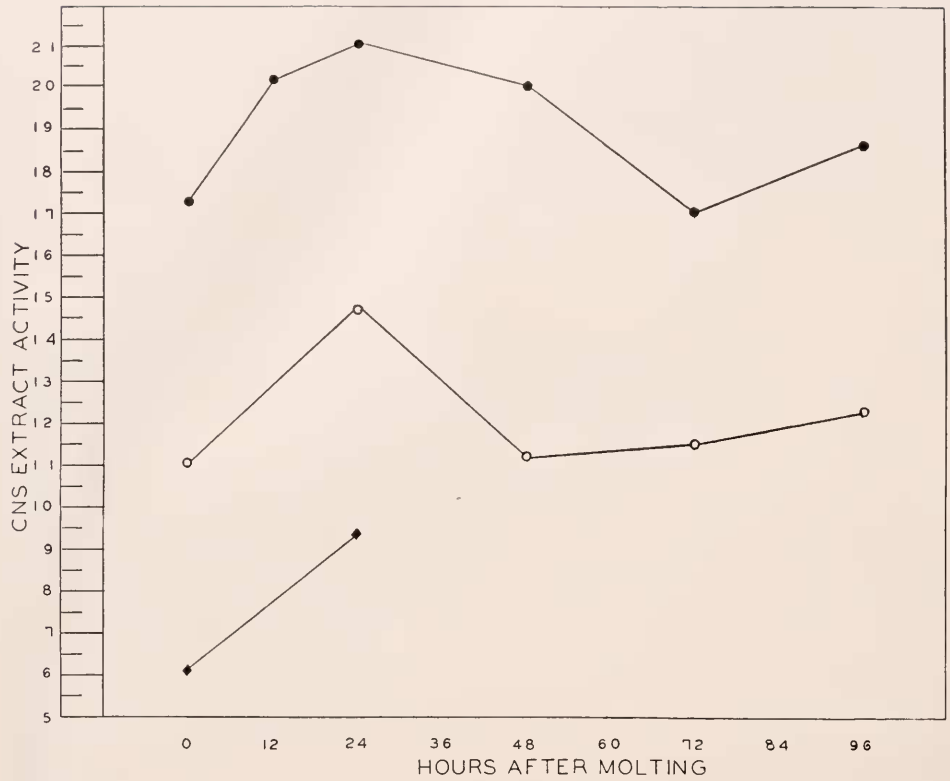

FIGURE 1. Average activity values of *Balanus eburneus* central nervous systems extracted at intervals following ecdysis and analyzed on eyestalkless fiddler crabs, *Uca pugilator:* solid circles, 1:1 dilution; open circles, 1:5 dilution; and solid diamonds, 1:10 dilution.

of activity at 24 hours after molting, followed by a decline in activity to the initial low point, and a slight increase in activity at 72 and 96 hours after molting. These data, when subjected to an analysis of variance, are highly significant at the 95% level.

Dilutions of the barnacle central nervous system extract of 1:10 also showed an activity which was low at the time of molting. This was followed by an increase at 24 hours after molting but insufficient data were available for additional time intervals to make further comparisons. Table II gives the total activity values for all dilutions of extracts used in the experiment.

Injection of barnacle opercular muscle extracts did not produce any reaction in the eyestalkless fiddler crab chromatophores. Day-to-day fluctuations in the chromatophore activity of the *Uca* controls (*Uca* injected with extracts of *Uca* eyestalks) were slight and never of sufficient magnitude to affect significantly the activity values obtained for the barnacle extracts.

Groups of barnacles from which central nervous systems were assayed represented a range of body weights from 24 mg. to 160 mg. Although this does not exceed a factor of 10 it was considered desirable to be sure that any consistent

TABLE II

Total activity values of central nervous systems of Balanus eburneus extracted at intervals within one molting period

	Hours after molting					
	0	12	24	48	72	96
1:1 Dilution	9.2	22.4	24.8	24.4	18.8	20.6
	21.8	22.6	23.8	23.6	12.4	20.8
	11.2	19.6	24.7	22.4	19.4	21.8
	19.8	17.8	9.6	24.0	22.4	15.0
	21.6	18.4	22.8	20.4	15.2	22.0
	20.2	20.2	18.6	12.8	11.2	9.0
	19.4	18.4	20.8	19.8	16.4	18.0
	11.2	24.0	20.0	11.6	20.4	21.6
	20.6	21.4	21.8	18.6		19.6
	11.4	20.4	17.0	23.4		
	21.3	14.6	24.8	20.2		
	19.6	22.0	24.4			
Average	17.3	20.2	21.1	20.1	17.0	18.7
1:5 Dilution	5.8		16.0	5.4	11.0	13.4
	14.6		15.2	13.0	9.6	13.0
	8.2		14.0	15.4	11.2	13.4
	7.2		11.0	10.4	9.8	12.6
	9.0		12.4	10.2	8.6	10.0
	9.6		14.4	9.8	14.8	12.6
	7.4		14.2	13.2	10.6	12.2
	11.3		17.6	13.4	11.8	12.4
	8.0		11.4	7.6	16.0	11.6
	14.8		14.3	14.0		
	13.5		18.2			
	12.3		6.0			
	18.4		10.8			
	9.6		18.2			
	12.8		17.8			
	16.4		18.8			
	13.2		17.6			
	11.4		15.6			
	3.6		16.4			
Average	11.0		15.5	11.2	11.5	12.4
1:10 Dilution	6.8		11.2			
	10.6		2.4			
	9.0		3.8			
	3.8		10.2			
	6.4		9.6			
	9.4		14.8			
	6.6		11.0			
	2.3		8.6			
	0.0		12.8			
Average	6.1		9.4			

change in the level of central nervous system activity was not solely a function of body size or weight. Accordingly, the data for central nervous system activity and the corresponding barnacle body weights were analyzed. The statistical treatment of these data indicates that body weight and activity of the chromatophorotropins within the central nervous system are independent.

Discussion

Although ecdysis in the barnacles, or Cirripedia, is analogous to the process described in considerable detail for the Decapoda, there is little evidence to date that the control of molting or the mechanisms of control in these two groups of Crustacea are similar. Attempts have been made to compare molting in the Cirripedia and in the Brachyura, either by studying possible sites of endocrine activity or storage organs or through investigations of physiological responses which may be similar. Most of our knowledge of the control of molting or the mechanisms of control in barnacles, however, is restricted to that gleaned from negative results.

Costlow and Bookhout (1958), studying the relationship between metabolic rate and the molting cycle in *Balanus amphitrite*, found that while variations in oxygen consumption did occur within any one molting period, there was no indication that an increase in respiratory rate occurred prior to molting, comparable to the two-fold increase described for some decapods (Roberts, 1957a, 1957b). Crisp and Patel (1960) described an endogenously regulated anecdysis in *Balanus balanoides* but Barnes (1962), working with the same species of barnacle, has suggested that the molting rhythm of this species is determined to a greater extent by environmental factors than endogenous factors. While temperature, diet, abundance of food, and reproductive state have been shown to affect the frequency of molting, at least one environmental factor which affects molting in some decapods, photoperiod, does not influence the molting frequency of barnacles (Costlow and Bookhout, 1956).

Evidence for a series of cyclic cellular changes within each molting period, comparable to those described for decapods by Drach (1939), has been scant. Thomas (1944) described the cyclic activity of the sublingual and subesophageal glands in *Balanus perforatus* and, while the cycles were concurrent, there was no indication that the activity of the glands was even indirectly involved in the control of molting. Costlow (1956) reported a secretory cycle in the shell-forming tissues in *Balanus improvisus* but cellular activity, as well as subsequent shell growth, was not associated with the 2–3-day molting frequency. Barnes and Gonor (1958a, 1958b) found neurosecretory cells in the central nervous system of several species of barnacles and gave a detailed description of the types of cells in *Pollicipes polymerus*, but did not associate the cyclic activity of these cells with the molting cycle. They also studied sections of whole bodies of *P. polymerus* but were unable to locate any storage organs for neurosecretory material and considered the possibility that discharge of such products may occur directly from the cells into the perineural blood sinus.

Sandeen and Costlow (1961) described one basic similarity which does exist between the Cirripedia and the Decapoda. They found two chromatophorotropins in the central nervous system of *Balanus eburneus*, *Chelonobia patula*, and *Lepas*

sp. which were similar in activity to the *Uca* black-dispersing substance and the *Palaemonetes* red-pigment-dispersing material. The function of these activators, especially in the absence of either chromatophores or eyestalks, or their possible role in molting of barnacles was not investigated, however.

In the present study it has been established that activity of the one chromatophorotropin within the central nervous system of *B. eburneus*, the *Uca* black-dispersing substance, is cyclic and that the cycle does conform to that demonstrated for the two–three-day molting cycle. The greatest changes in level of activity are those at the time of molting and at 48 hours after molting, when the activity is lowest, and at 24 hours after molting when the activity is highest (Fig. 1). The slight increases in activity at 72 and 96 hours after molting are not statistically significant but do deserve some comment. Normally, at the temperatures under which the experimental barnacles were maintained (23°–26° C.), the frequency of molting is every 2–3 days. Thus, at 72 hours after the initial molt the barnacle would normally have begun the next molt and the activity of the chromatophorotropin would be low (Fig. 1). At 96 hours after the first molt the animal would normally have completed the next molt and the activity might be expected to equal the peak which corresponds to that shown for the 24-hour interval following ecdysis. In animals which do not initiate the mole or complete it within the regular 2–3-days frequency the activity of the central nervous system extracts never attained the peak described for the 24-hour interval following molting.

While there is no evidence that the chromatophorotropins of the central nervous system of barnacles are directly associated with the control of molting, the present study does suggest several possible functions and relationships. If we accept the suggestion of Barnes and Gonor (1958b) that in the absence of a storage site the neurosecretory products of the central nervous system are released directly into the hemolymph, it is possible to carry the concept of hormonal control of molting in the barnacles one step further. In the absence of a storage site the neurosecretory products are accumulated in the central nervous system itself. Secretory activity of the cells would increase following molting and reach a peak at 24 hours following ecdysis, the point of highest activity and presumably the highest concentration. This would be followed by a gradual release of the material into the hemolymph during the next 24-hour period until the initial low level was again reached in the central nervous system. If these products were to include a molt-accelerating substance, the release of material into the hemolymph between 24 and 48 hours following molting would serve to stimulate those additional physiological processes which are prerequisite to the actual process of molting. Following ecdysis the cycle would be repeated, continuing the 2–3-day frequency of molting.

In barnacles which have not molted for a second time within 96 hours following the previous molt, the absence of an increase in activity of the chromatophorotropins of the central nervous system, comparable to that found at 24 hours after molting, can be attributed to at least one of two things: (1) secretory activity within the central nervous system may have been insufficient to accumulate products equal to the titer found during the previous interval and thus molting is not initiated, or (2) the general physiological level of the animal, or the general metabolic state, had been reduced by environmental factors in the laboratory, and all functions, including molting, were retarded. Because the central nervous system extract of

any one barnacle can only be analyzed for one time interval within any one molt, it is not possible to follow the entire cycle of activity of the chromatophorotropins within any one barnacle.

Further studies obviously are needed to delineate the various components of the central nervous system extracts and determine, either by injection or implantation, if any of the various fractions can affect molting in the Cirripedia or the Brachyura.

Summary and Conclusions

1. The central nervous system of the barnacle, *Balanus eburneus,* was removed at known intervals following ecdysis, extracted in sea water, and assayed by injecting into eyestalkless *Uca pugilator* to determine if the barnacle chromatophorotropins exhibited a cyclic activity associated with molting.

2. A cyclic pattern of activity was observed within one intermolt period. The changes in activity of the *Uca* black-pigment-dispersing substance, highly significant at the 95% level, were the low concentrations immediately following molting and 48 hours after ecdysis and the high concentration which occurred at 24 hours after molting. In barnacles which did not molt again within the usual 72–96-hour period following the first molt, the level of activity of the central nervous system extracts remained low. Body weight and the activity of the central nervous system extracts were found to be independent.

3. The hypothesis is presented that in the absence of a storage organ comparable to the sinus gland of Brachyura, neurosecretory products originating from the central nervous system are released directly into the blood. The release of these products following the period of greatest concentration, 24 hours after molting, stimulates the physiological processes and cellular changes which culminate in ecdysis.

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